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# SEMI-ANNUAL PROGRESS REPORT

Report Prepared By: Dr. M. Michael Sigel  Date: June 25, 1952

NR: 130-706  For Period: 1/1/52 to 6/30/52

CONTRACT: N8onr-72601

PRESENT ANNUAL RATE:

CONTRACTOR: The Children's Hospital of Philadelphia

PRINCIPAL INVESTIGATOR: M. Michael Sigel, Ph. D.

Assistants: Anthony J. Girardi, Graduate Student and United States Public Health Fellow
Emma G. Allen, Graduate Student
Ralph Pollikoff, Graduate Student
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TITLE OF PROJECT: "Studies on the Psittacosis-Lymphogranuloma Venereum Group"

Objectives:

1. To search for a specific complement fixing antigen. At present, there is no satisfactory serologic procedure available for differential diagnosis of infections with members of this group.

2. To study mode of growth and multiplication and other biologic characteristics of this group of agents. These studies may have a bearing on the problem listed under (1). Furthermore, knowledge obtained from these studies may help define the position of these agents in the microbial world.

3. To study the effect of therapeutic agents (antibiotics) on these viruses and on the diseases caused by them.

Abstract (or Summary) of Results:

A. Since Start of Project:

Work on the Complement-Fixing Antigens.

1. Preliminary studies involving large series of complement fixation tests using the systems and reagents listed below have occasionally shown antigenic differences, but these differences have not been consistent.

a. Crude antigens of members of this group prepared in chick embryos.

b. Antigens and fractions obtained from differential centrifugation and chemical treatment or extraction.

c. Antisera prepared in hamsters and guinea pigs.

d. Sera from patients infected with viruses of this group.

e. Antisera previously exposed to several antigens in an attempt to absorb group specific antibodies.

Thus, although these experiments have failed to establish the existence of a specific complement fixing antigenic fraction, it is our opinion that such a fraction does exist and this problem will be pursued further.

2. In view of the difficulties encountered with the specific neutralization of the MP virus, an investigation of the factors controlling the neutralization test was carried out. The LCM virus and anti-LCM human sera were employed in this investigation.

3. Regarding the complement fixation tests, we confirmed the observations of Heidelberger and Kabat on the superiority of veronal buffer over phosphate buffer. Veronal buffer is now used in place of phosphate in all complement fixation tests performed in this laboratory.

Work on Biological Properties of the Psittacosis-Lymphogranuloma Group.

1. The phenomenon of auto-interference was demonstrated in experiments using meningopneumonitis (MP) virus -- a member of the psittacosis-lymphogranuloma group. MP virus inactivated by ultraviolet light inhibited the growth of active MP virus. These studies will be continued.

2. An extensive study on the preservation of a member of this group (meningopneumonitis) showed that the optimal temperature of preservation was about -70 C and that addition of certain protective substances facilitated preservation of viral activity at several temperatures. Skim milk was found to be the best of all substances tested with this virus.

3. Infectivity of the MP virus was assayed by several methods (development of elementary bodies in the chick embryo, death of embryos and death of mice inoculated by the cerebral route). Assay in mice by the cerebral route was found to be the most satisfactory method (because of greater regularity of results and simplicity), although titration in embryos, based on development of elementary bodies, was the most sensitive procedure.

4. Studies on the development of the MP virus in the chorioallantois have so far revealed the following:

In the allantoic fluid in the egg, the changes in infectivity (amount of infective virus) during the first 24 hours corresponded closely to the changes in allantoic fluid in vitro at 36 C, indicating that only a relatively small amount of virus was necessary to initiate infection of the susceptible tissue (membrane) and that the decrease in viral activity was due to thermal inactivation. In the membrane, at least three phases were seen: 1) Increase in infectivity in the first few hours probably due to prolonged adsorption of virus from the allantoic fluid; 2) decrease in infectivity, and 3) sharp increase in infectivity due to development of new virus. This phase of the work is being continued.

5. Preliminary experiments indicated that multiplication of MP virus did not stop the growth of the host cells (cells of the allantoic membrane).

6. Experiments on changes in infectivity of MP virus in membranes removed at several intervals from the egg and placed in tissue culture suggested the following explanations for the observed phenomena:

a. That the initial increase in the membrane is due to adsorption.

b. That the subsequent decrease is probably due to change in state of virus (transformation from infectious to non-infectious).

7. The phenomenon of diminishing infectivity following adsorption of virus to the membrane ("the eclipse phenomenon" or "the latent period") and preceding appearance of new active virus which was described previously with MP virus in the allantois has now also been demonstrated for the virus in tissue culture (Simm's serum ultrafiltrate) and on the choriollantoic membrane.

8. A preliminary experiment failed to show interference between MP virus and the PR8 influenza virus.

9. Work was commenced on cultivation of MP virus in cancer cells. The early phases of this work dealt with preparations of mouse ascites tumors, preliminary determinations of survival or growth of the tumor cells in tissue culture and initial studies on multiplication of the virus in these cells.

a. There was no difficulty in producing ascites and solid tumors in mice.

b. The tumor cells can survive for considerable length of time in Simm's serum ultrafiltrate but definite evidence of their multiplication in this medium is still lacking.

c. Although MP virus was found in ascites tumors as long as 144 hours after inoculation, proof of multiplication of this virus in tumor cells is still missing.

Effect of Therapeutic Agents on these Viruses.

1. Patients with lymphogranuloma venereum showed a variable response to treatment with aureomycin. The most encouraging results were obtained in patients with acute inguinal adenitis and those with comparatively early rectal infections. In two instances, there was a reversal in serologic reaction. These studies are being continued.

2. Experiments on the fate of aureomycin in the chick embryo have shown that aureomycin may still be present in the allantoic fluids after 120 hours, in the allantoic membranes up to 72 hours, but that it disappears from the plasma in 1-4 hours.

3. Preliminary determinations on the growth of MP virus in the embryo treated with 1 mg. of aureomycin at several intervals after infection indicated that the shorter the time interval, the lower the titer of virus in allantoic membranes when determined at 30 hours.

4. Some experimental data suggested that aureomycin may affect the virus in the host tissue.

5. Experiments on the effect of aureomycin on the amount of virus which develops in 30, 50 and 72 hours in the allantois indicated that the earlier the administration of the drug, the less virus developed at these intervals. The results are not regarded as definitive and further experiments are in progress.

6. The possible reversing effect of citrate on the activity of aureomycin reported in the literature was tested. It was found (one experiment) that 0.02 M NaCit had no inhibitory or reversing effect on aureomycin, whereas 0.2 M NaCit stopped growth of virus by itself. The mechanism of this activity was not determined. It remains to be shown whether the absence of virus from the tissue culture treated with NaCit was the result of actual antiviral activity or merely the result of possible toxicity of this substance in this concentration leading to death of the tissue.

7. Experiments on virus in membranes treated in ovo with aureomycin at several intervals and subcultured in tissue culture furnished further evidence that action of the drug on the virus took place in the tissue.

8. When embryos were treated with 1 mg. of aureomycin at times -1, 0, 2, 6 and 26 hours after injection of MP virus, 63-90% of the embryos were alive up to one day of hatching as compared with 10% of the controls.

9. It was also found that aureomycin in concentration of 0.0075 mg./0.03 cc. (maximum amount that may be present in embryonic fluids after injection of 1 mg. into embryo) had no significant effect on intracerebrally inoculated MP virus in mice. Although some animals survived a day or two longer, the titer of the virus (the $LD_{50}$) remained the same. Thus, residual aureomycin in preparations assayed in mice by the intracerebral route should not introduce an error (i. e., it should not bring about false diminution in infectivity titer).

B. During Current Report Period:

Work on Antigens (Search for Specific Complement Fixing Antigen).

1. Protein and carbohydrate fractions of MP virus grown in chick embryos were prepared by the method of Sevag.

2. Although both fractions reacted in the complement fixation test, the protein fraction appeared to be more active. Intensive work with these fractions is now in progress.

3. Absorption of antisera with boiled MP antigen (an antigen in which the specific component has presumably been destroyed) removed to a large extent "group reactivity" leaving behind most of the "specific activity". These findings appear to be very significant. They are being followed up.

Work on Biological Properties of the Psittacosis-Lymphogranuloma Venereum Group.

1. One phase of this study was completed. It may be summarized as follows:

a. The pattern of growth of meningopneumonitis virus *in vitro* seemed to be similar to that occurring *in ovo* and thus the initial stages of development, the adsorption and the latent periods, were investigated by the use of tissue culture procedures.

b. The initial increment of infectivity in allantoic membrane suspensions following virus inoculation *in ovo* was due to prolonged adsorption of virus and not to immediate virus reproduction. The length of the adsorption period varied with the virus dilution employed.

c. The reduction of virus titer in allantoic membrane suspensions subsequent to adsorption was due to a change of infectious virus to a non-infectious form and this seemed to be a part of the normal developmental cycle of the virus.

2. In connection with the studies on cultivation of MP virus in cancer cells, the following observations were made:

a. Krebs ascites tumor cells survived longer in complete tissue culture (Tyrode, + human serum + chick embryo extract) than in Simm's fluid.

b. MP virus was found to inhibit or prevent the development of this tumor in mice.

c. We have as yet no definite evidence, however, that MP virus multiplies in the tumor cells. Studies aimed at elucidation of this point are now in progress, including attempts at breaking up tumor cells.

d. A new type of tissue disintegrator (manufactured by H. Mickle Company, Hampton, Middx., England) was found to break up all tumor cells in about 30 minutes without producing any decrease in the infectivity of MP virus.

Effect of Therapeutic Agents on These Virus

1. Work with lymphogranuloma venereum patients was moving very slowly because of the difficulties enumerated in the last report (see Supplemental Report).

2. One phase of the studies with antibiotics in chick embryos infected with MP virus was completed and may be summarized as follows:

a. Data indicated that the drug had no *in vitro* effect on the virus particle itself -- that is, aureomycin was not capable of altering the extracellular virus.

b. The drug appears to affect virus multiplication by causing an extension of the "latent" period (see work on Biological Properties, 1a and 1c).

c. It was found that complete inhibition of growth during the time interval corresponding to the first cycle of growth occurred only if aureomycin was administered during the first 6-8 hours of virus growth. This would seem to indicate that after this time virus synthesis had passed beyond the process or stage in development which could be blocked by the drug.

d. The chief role of the antibiotic appeared to be one of virostasis, for the virus was able to resume its growth process when a critical low level of the drug in the allantoic membrane was reached.

e. In connection with prolonged survival of treated embryos, it is especially interesting that virus was not found in the brains of treated embryos up to at least 192 hours after inoculation of virus. This is in contrast with the findings in allantoic membranes and livers of such embryos; these organs showed virus at 120 and 144 hours, respectively. (In untreated controls, virus appeared in membranes at 24 hours, in the liver at 48 hours and in the brain at 72 hours.)

PLANS FOR THE FUTURE:

Immediate:

Continuation of work on pattern of multiplication on chorioallantoic membrane, in tissue culture and cancer cells (morphology, etc.).

Continuation of work on the interference phenomenon.

Continuation of work on effect of therapeutic agents (in patients and chick embryos). Intensification of work with patients if proposed study outside Continental United States of America becomes possible.

Studies on absorption of antisera with fractions of MP antigen.

Studies on protein and carbohydrate fractions of MP virus grown under various conditions.

Long Range:

Follow-up of LGV patients treated with antibiotics.

Search for specific complement fixing antigen.

Work on multiplication of these agents in suspended (isolated) cell tissue culture and cancer cells.

REPORTS AND PUBLICATIONS:

1. The paper entitled "Preservation of Viruses of the Psittacosis-Lymphogranuloma Venereum Group and Herpes Simplex under Various Conditions of Storage" by Emma G. Allen, Ben [illegible], Anthony J. Girardi, T. F. McNair Scott and M. Michael Sigel was published in The Journal of Bacteriology, Vol. 63, pp. 369-376. 1952

2. A paper entitled "Factors Influencing the Neutralization of Lymphocytic Choriomeningitis Virus" by Ralph Pollikoff and M. Michael Sigel was presented at the Boston meeting of the Society of American Bacteriologists in April.

3. The paper entitled "Studies on the Psittacosis-Lymphogranuloma Group. II. A Non-Infectious Phase in Virus Development Following Adsorption to Host Tissue" by Anthony J. Girardi, Emma G. Allen and M. Michael Sigel was accepted for publication in The Journal of Experimental Medicine.

4. A paper entitled "Studies on the Psittacosis-Lymphogranuloma Group. III. The Effect of Aureomycin on the Propagation of Virus in the Chick Embryo" by Emma G. Allen, Anthony J. Girardi, M. Michael Sigel and Morton Klein is to be published.

Other Papers Published by Responsible Investigator:

"Leptospiral Meningitis. Report of a Case Resembling Swineherd's Disease Due to Leptospira Pomona" by T. B. Krouse and M. M. Sigel. Ind. Med. & Surgery, 21, 121, 1952.

"Survey of Epidemic Typhus Antibody Levels in Bloods of Individuals Born in Eastern and Central Europe and the United States" by M. M. Sigel, L. B. Weiss, N. Blumberg and J. C. Doane. Am. J. Med. Sci., 223, 409, 1952.

"Coxsackie Viruses and Human Disease" by M. M. Sigel. Advances in Medicine and Surgery from the Graduate School of Medicine of University of Pennsylvania. W. B. Saunders Company. P. 372, 1952.

"Influence of Age on Susceptibility to Virus Infections" by M. M. Sigel. Annual Review of Microbiology. In press.

SUPPLEMENTAL REPORT

1/1/52 to 6/30/52

NR: 130-706

1. Dr. Anthony Girardi and Dr. Emma Allen received their Ph. D. degrees at the June Commencement, 1952, at the University of Pennsylvania. The research work and theses of these students were part of the research program supported by the Office of Naval Research. It gives me great pleasure to acknowledge the generous help of ONR, not only in the pursuance of our research program, but also in the training of scientists.

2. The summary of our findings listed under Section B, Work on Biological Properties (2b), deserves special mention. Although other viruses have previously been shown to prevent tumor formation in experimental animals, the virus used by us, MP, may be especially useful in this connection because it is susceptible to the action of antibiotics, such as aureomycin (see paper III on our Series, "Studies on the Psittacosis-Lymphogranuloma Group"), and may thus be controlled by means of aureomycin.

3. In view of the difficulties encountered in the program of aureomycin therapy of patients in Philadelphia (see last Supplemental Report) we have attempted to find an area where this problem could be studied more advantageously. Thus far, we have been unsuccessful in locating such an area. You are familiar with the communications pertaining to this with the Bureau of Medicine and Surgery. In addition, we have contacted the Department of State, the Department of Interior, and a number of territorial Commissioners of Health. The State Department did not consider this project feasible at this time under the Point-4 Program. As regards the territories, LGV is either non-existent or of very low incidence in Alaska, American Samoa, Guam and Puerto Rico. We are still hoping to hear from the Commissioners in Honolulu, Oahu and the Virgin Islands. Inquiry was also sent to Jamaica. We hope that UNWHO will be interested in this study. It certainly has a bearing on Public Health in port cities. Recently, we had the opportunity to test specimens from sailors of foreign vessels (one from Dakar, North Africa and two from Greece).

4. The findings described under "Work on Biological Properties" (1a, b, c) indicate that agents of the psittacosis-lymphogranuloma-ornithosis group appear to multiply in a manner analogous to that reported in recent years for "true" viruses such as bacteriophage, influenza, PVM, etc.